

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

p53 Arg72Pro and MDM2 309 SNPs in hereditary retinoblastoma

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/89072> since 2020-06-17T15:20:06Z

Published version:

DOI:10.1038/jhg.2011.82

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)



UNIVERSITÀ DEGLI STUDI DI TORINO

This is an author version of the contribution published on:

Questa è la versione dell'autore dell'opera:

[J Hum Genet. 2011 Sep;56(9):685-6. doi:10.1038/jhg.2011.82]

The definitive version is available at:

La versione definitiva è disponibile alla URL:

[<http://www.nature.com/jhg/journal/v56/n9/full/jhg201182a.html>]

p53 Arg72Pro and MDM2 309 SNPs in hereditary retinoblastoma

Maria Carmela Epistolato 1, Vittoria Disciglio 1, Gabriella Livide 1, Paola Berchialla 2, Annabella Marozza 1, Mariangela Amenduni 1, Theodora Hadjistilianou 3, Sonia De Francesco 3, Antonio Acquaviva 4, Paolo Toti 5, Francesco Cetta 6, Francesca Ariani 1, Mario De Marchi 7, Alessandra Renieri 1, Daniela Giachino 7.

1 Medical Genetics, Department of Biotechnology, University of Siena, Siena, Italy

2 Department of Public Health and Microbiology, University of Torino, Torino, Italy

3 Ophthalmological Science and Neuroscience, Siena General Hospital, Siena, Italy

4 Pediatrics Department, University of Siena, Siena, Italy

5 Department of Human Pathology and Oncology, University of Siena, Siena, Italy

6 Department of Surgery, University of Siena, Siena, Italy

7 Medical Genetics Unit, Department of Clinical and Biological Sciences, University of Torino, Torino, Italy

Corresponding author:

Daniela Giachino M.D.
Medical Genetics Unit
Department of Clinical and Biological Sciences
University of Torino
Regione Gonzole 10
10043 Orbassano (TO)
Tel +39 011 6705465
Fax +39 011 6705428
Email daniela.giachino@unito.it

Key words

Hereditary Retinoblastoma, Single Nucleotide Polymorphisms, MDM2, p53

Abstract

The tumor suppressor p53 and its negative regulator MDM2 play crucial roles in a variety of cellular functions such as the control of the cell cycle, senescence, genome stability and apoptosis, and are frequently deregulated in carcinogenesis. Previous studies have highlighted the contribution of the common functional polymorphisms p53 p.Arg72Pro and *MDM2* 309SNP to the risk of both common cancers and Li-Fraumeni syndrome. Their possible role in retinoblastoma has recently been addressed by Castera et al, who however only studied the *MDM2* 309SNP. Here, for the first time, we analyzed both SNPs in a case-control study of 111 Italian hereditary retinoblastoma patients. We found a significant association of the p53 Pro/Pro genotype with the disease (OR=3.58, p= 0.002). The *MDM2* 309SNP showed a weak negative association of allele G that deserves further investigation. These findings further support the hypothesis that genetic variability of the p53 pathway contributes to the individual susceptibility to retinoblastoma, as shown for Li-Fraumeni syndrome and a variety of non hereditary cancers.

Text

Retinoblastoma (RB, OMIM#180200), the most common primary intraocular malignancy in children, affecting 1: 14 000 to 1:22 000 live births, is caused by biallelic inactivation of *RB1*¹. In about 40% of patients a germ-line mutation inactivates one allele and a somatic one the second allele (hereditary RB), while in non-hereditary RB both alleles are inactivated somatically. Although most children with hereditary RB show multiple bilateral tumors, a significant proportion of carriers remain unaffected or only develop unilateral tumors, or benign retinomas². Penetrance and expressivity of hereditary RB may depend on the type of inherited mutation, and can vary even within families and among patients with identical mutations³⁻⁵. This indicates a role of modifiers that may affect genome stability to favor the occurrence of somatic mutations, and/or the apoptotic pathway to induce loss or maintenance of the mutated retinoblasts.

The p53 pathway, the master control system of these processes, is controlled by a feedback autoregulatory loop in which p53 transcriptionally activates MDM2, that in turn acts as a negative regulator by promoting the proteolytic degradation of p53. Interestingly, this circuit can also target pRB to degradation and in physiological condition controls the cell cycle and apoptosis of the retinal cone precursors, from which the RB cell lineage originates⁶. In RB p53 is not usually mutated nor is MDM2 amplified. Rather, the expression of MDM2 is highly induced and represses p53⁶, thus leaving ample space for constitutional polymorphism of p53 and/or MDM2 to affect the fate of the RB lineage.

The 72Pro allele of p53 SNP (p.Arg72Pro, rs1042522:G>C) features a reduced proapoptotic activity compared with the 72Arg allele, and together with its more efficient induction of the *MDM2* gene and other p53 targets eventually results in attenuation of the p53 pathway⁷.

The G allele of *MDM2* 309SNP (c.14+309T>G, rs2279744:T>G), adjacent to p53-responsive elements of the inducible intronic P2 promoter, activates *MDM2* transcription⁸ and results in overdegradation of p53⁹. Several studies have highlighted the risk-modifying effect of these polymorphisms in common cancers and the rare Li-Fraumeni syndrome caused by germinal *TP53* mutations^{10,11,12}. The possible role of the *MDM2* 309SNP in RB has only recently been addressed through a family-based approach¹³.

For the first time we here investigated both SNPs. We took advantage of a sample of 111 RB patients with known germinal *RB1* mutation (50% M, 93 bilateral cases, median age at diagnosis 22.75 months (IQR 10.29-37.79)) from the Italian DataBase of Retinoblastoma, referred to the Medical Genetics Unit of Siena since 2003 for germline *RB1* mutations testing. Written consent was obtained from all subjects or their legal tutors. Controls were 307 unrelated Italian individuals.

The two SNPs were genotyped on blood DNA using Pyrosequencing®, according to standard protocols. PCR and sequencing primers were designed using the PSQ Assay Design Software version 1.0.6 (Biotage AB, Uppsala, Sweden, now Qiagen) and are available on request. Pyrograms were analyzed using the PSQ96MA 2.0.2 software. The association of each SNP and of the combined genotypes were analyzed in a case-control design using logistic regression with Firth's bias reduction procedure to assess their effect on the disease risk.

Allele frequencies of the p53 SNP were Arg 0.68 and Pro 0.32 among RB patients vs 0.78 and 0.22 in controls, those of the *MDM2* 309SNP T 0.66 and G 0.34 vs 0.59 and 0.41. The genotype frequencies of patients and controls were in Hardy-Weinberg equilibrium for both polymorphisms. The Pro/Pro genotype was significantly disease-associated compared with the Arg/Arg (Table 1a); the heterozygous Arg/Pro was also increased but not significantly. The *MDM2* 309SNP showed a weak negative association of the G allele; the GT genotype was significant within the Arg/Arg subgroup (Table 1b).

For the first time we show that the association of the Pro/Pro genotype, well known in other cancers, also holds true in hereditary RB. p53 has a central role as tumor suppressor but is not mutated in RB¹⁴⁻¹⁶. It has been suggested that RB precursor are intrinsically apoptosis-resistant cells¹⁷. However, there is evidence in mice and humans that the p53 pathway plays a central role in regulating the progression of RB cells from quiescent retinoma to overt disease^{2,18}. Two features of the p53 72Pro allele can be hypothesized to contribute to this outcome i.e. its increased transcriptional activity on target genes and the weaker interaction with the MDM2 protein, the first by enhancing MDM2 hyperexpression, and the second by downregulating the mitochondrial control of the apoptotic pathway⁷.

On the other hand, our finding of a weak negative association of the *MDM2* G allele is in contrast with reports of its preferential transmission to RB patients¹³, modifier role in Li-Fraumeni syndrome^{9,19} and association with common cancers²⁰. Nevertheless our findings seem plausible, considering that the association only holds in p53-mutated cancers²⁰. If confirmed in other independent studies, the pattern we report here of positive association of p53 Pro/Pro and negative association of *MDM2* GG would depict a new scenario with different functional and therapeutic implications.

REFERENCES

1. Knudson, A.G. Hereditary predisposition to cancer. *Ann N Y Acad Sci* **833**, 58-67 (1997).
2. Dimaras, H., Khetan, V., Halliday, W., Orlic, M., Prigoda, N.L., Piovesan, B. et al. Loss of RB1 induces non-proliferative retinoma: increasing genomic instability correlates with progression to retinoblastoma. *Hum Mol Genet* **17**, 1363-72 (2008).
3. Klutz, M., Brockmann, D. & Lohmann, D.R. A parent-of-origin effect in two families with retinoblastoma is associated with a distinct splice mutation in the RB1 gene. *Am J Hum Genet* **71**, 174-9 (2002).
4. Lohmann, D.R. & Gallie, B.L. Retinoblastoma: revisiting the model prototype of inherited cancer. *Am J Med Genet C Semin Med Genet* **129C**, 23-8 (2004).
5. Sampieri, K., Hadjistilianou, T., Mari, F., Speciale, C., Mencarelli, M.A., Cetta, F. et al. Mutational screening of the RB1 gene in Italian patients with retinoblastoma reveals 11 novel mutations. *J Hum Genet* **51**, 209-16 (2006).
6. Xu, X.L., Fang, Y., Lee, T.C., Forrest, D., Gregory-Evans, C., Almeida, D. et al. Retinoblastoma has properties of a cone precursor tumor and depends upon cone-specific MDM2 signaling. *Cell* **137**, 1018-31 (2009).
7. Dumont, P., Leu, J.I., Della Pietra, A.C. 3rd, George, D.L. & Murphy, M. The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. *Nat Genet* **33**, 357-65 (2003).
8. Iwakuma, T. & Lozano, G. MDM2, an introduction. *Mol Cancer Res* **1**, 993-1000 (2003).
9. Bond, G.L., Hu, W. & Levine A. A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. *Cell* **119**, 591-602 (2004).
10. Fang, S., Krahe, R., Lozano, G., Han, Y., Chen, W., Post, S.M. et al. Effects of MDM2, MDM4 and TP53 codon 72 polymorphisms on cancer risk in a cohort study of carriers of TP53 germline mutations. *PLoS One* **5**, e10813 (2010).
11. Wu, C.C., Krahe, R., Lozano, G., Zhang, B., Wilson, C.D., Jo, E.J. et al. Joint effects of germ-line TP53 mutation, MDM2 SNP309, and gender on cancer risk in family studies of Li-Fraumeni syndrome. *Hum Genet* (2011).
12. Francisco, G., Menezes, P.R., Eluf-Neto, J. & Chammas, R. Arg72Pro TP53 polymorphism and cancer susceptibility: A comprehensive meta-analysis of 302 case-control studies. *Int J Cancer* (2010).
13. Castéra, L., Sabbagh, A., Dehainault, C., Michaux, D., Mansuet-Lupo, A., Patillon, B. et al. MDM2 as a modifier gene in retinoblastoma. *J Natl Cancer Inst* **102**, 1805-8 (2010).
14. Kato, M.V., Shimizu, T., Ishizaki, K., Kaneko, A., Yandell, D.W., Toguchida, J. et al. Loss of heterozygosity on chromosome 17 and mutation of the p53 gene in retinoblastoma. *Cancer Lett* **106**, 75-82 (1996).
15. Gallie, B.L., Campbell, C., Devlin, H., Duckett, A. & Squire, J.A. Developmental basis of retinal-specific induction of cancer by RB mutation. *Cancer Res* **59**, 1731s-1735s (1999).
16. Sampieri, K., Amenduni, M., Papa, F.T., Katzaki, E., Mencarelli, M.A., Marozza, A., et al. Array comparative genomic hybridization in retinoma and retinoblastoma tissues. *Cancer Sci* **100**, 465-71 (2009).
17. Chen, D., Livne-bar, I., Vanderluit, J.L., Slack, R.S., Agochiya, M., Bremner, R. Cell-specific effects of RB or RB/p107 loss on retinal development implicate an intrinsically death-resistant cell-of-origin in retinoblastoma. *Cancer Cell* **5**, 539-51 (2004).
18. Nork, T.M., Poulsen, G.L., Millecchia, L.L., Jantz, R.G. & Nickells, R.W. p53 regulates apoptosis in human retinoblastoma. *Arch Ophthalmol* **115**, 213-9 (1997).
19. Bougeard, G., Baert-Desurmont, S., Tournier, I., Vasseur, S., Martin, C., Brugieres, L. et al. Impact of the MDM2 SNP309 and p53 Arg72Pro polymorphism on age of tumour onset in Li-Fraumeni syndrome. *J Med Genet* **43**, 531-3 (2006).

20. Hu, Z., Jin, G., Wang, L., Chen, F., Wang, X., Shen, H. MDM2 promoter polymorphism SNP309 contributes to tumor susceptibility: evidence from 21 case-control studies. *Cancer Epidemiol Biomarkers Prev* **16**, 2717-23 (2007).